GOLD NANO-CONJUGATES: A LEAP IN CANCER TREATMENT

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ABSTRACT: The use of nanoparticles as drug delivery and targeting agents has led to immense progress in the field of biomedicine and diagnostics. Likewise gold nanoparticles can be conjugated with various molecules, for instance, to plasmid DNA for gene therapy, to various drugs for antibacterial and anticancer properties, to several antibiotics and peptides for immunological and diagnostic purposes. The review encompasses various fields in which the gold nanoparticle conjugates have emerged as a promising tool for effective therapeutics and diagnosis. The article addresses a brief knowledge about the subject available to readers about various gold nanoparticle conjugates that have been researched. Although more work is needed to establish the toxicity concerns yet these particles have proved to be a promising therapy for the diagnosis and treatment of various diseases, especially cancer. With the cancer cells becoming resistant to anti-cancer drugs, the use of gold nanoparticles for the enhanced delivery and increased action of anti-cancer drugs has paved a new way to battling against cancer.

INTRODUCTION: Derived from the Greek root “nanos”, the word “nano” means small and is used to prefix for one billionth part (10⁻⁹). American Society for Testing and Materials(ASTM international 2006) defined Nanoparticles as those particles which have two or more than two dimensions and are in the size range of 1–100 nm.¹ These Nanoparticles differ from their bulk materials in having special and enhanced physical and chemical properties. This is due to their large, reactive and exposed surface area and quantum size effect as a result of specific electronic structures.² Nanoparticles are widely used in electronics, biomedicine and various other fields. In medicine they are used for diagnostic and therapeutic functions.

Due to their very small size, Nanoparticles have found immense use as targeted drug delivery agents. They are currently being used in oncology to serve multifunction, which are, diagnosis, drug delivery, gene delivery, phototherapy, chemotherapy, imaging mechanisms, and bioseparation.³,⁴

They can be synthesized from different organic or inorganic materials or a hybrid of organic and inorganic materials. Inorganic platforms are most important for diagnosis and simultaneous therapy due to their easy modification, stability and high drug loading capacity.⁵

Metal Nanoparticles, especially Gold Nanoparticles have tremendous role in the field of medicine due to their large surface area and unique optical properties. Unlike the bulk form of gold which is yellow in colour and inert in nature, gold Nanoparticles are wine red in colour and have anti-oxidant properties. Gold Nanoparticles show various sizes ranging from 1nm to 8 μm and are
found in different shapes, like as spherical, tetrahedral, sub-octahedral, octahedral, nanotriangles decahedral, icosahedral multiple twined, irregular shape, nanoprism, hexagonal platelets and nanorods.\(^2\)\(^,\)\(^6\)

Difference in shape and size impart unique electric and magnetic properties to gold Nanoparticles, which has led to many biochemical applications.\(^7\)

For instance, nanorods, nanoshells, and other gold nanocrystals can absorb light in the near infrared (NIR) region. This makes them helpful for biological purposes, given the high transparency of tissues to light of that wavelength.\(^7\) Huang et al., while working on gold nanoparticles of various sizes demonstrated that ultra small GNP (smaller than 10 nm) showed advantages over GNP with larger sizes in terms of penetration and localization into breast cancer cells and tumors in mice.\(^8\)

**TABLE 1: SHAPES OF GOLD NANOPARTICLES AND THEIR APPLICATIONS**\(^2\)

<table>
<thead>
<tr>
<th>Shape</th>
<th>Size</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano rod</td>
<td>2-5 nm</td>
<td>Drug delivery and photothermal therapy</td>
</tr>
<tr>
<td>Hollow particle</td>
<td>25 nm</td>
<td>Photo-electronics, catalysis and cancer therapy</td>
</tr>
<tr>
<td>Triangular particle</td>
<td>3.85-7.13 nm</td>
<td>Highly effective against E. coli and K. pneumonia</td>
</tr>
<tr>
<td>Faceted particle</td>
<td>50-100 nm</td>
<td>Effective, reproducible, and stable large area substrates for NIR SERS</td>
</tr>
<tr>
<td>Nanocube</td>
<td>50 nm</td>
<td>Field enhanced applications and refractive-index Sensing</td>
</tr>
<tr>
<td>Nancage</td>
<td>50 nm</td>
<td>Effective molecular contrast agent for nonlinear endomicroscopy imaging and in</td>
</tr>
<tr>
<td>Nanobelt</td>
<td>Thickness<del>80 nm, Width</del>20 μm, Length~0.15 m</td>
<td>Strain sensors</td>
</tr>
<tr>
<td>Branched particle</td>
<td>90 nm</td>
<td>Substrates for SERS-based imaging of kidney cells</td>
</tr>
</tbody>
</table>

2. **Advantages of gold nanoparticles:**

1. Gold has higher absorption coefficient than iodine (due to its higher atomic number and electron density). Therefore gold Nanoparticles are advantageous over iodine-based agents in X-ray and CT scan imaging as molecular probes.\(^9\)

2. Non-cytotoxic \(^2\)

3. Gold Nanoparticles can be readily modified with targeting molecules and specific biomarkers due to their large surface area. They can bind with wide range of organic molecules and used as therapeutic agents or vaccine carrier.\(^10\)

4. PEG-coated Gold Nanoparticles have been found to have radiosensitizer role in cancer radiation therapy. In vitro and in vivo radiosensitization studies have shown that 12.1 and 27.3 nm PEG-coated GNP have greater sensitization effects and can cause a significant decrease in cancer cell survival after gamma radiation.\(^11\)

Gold Nanoparticles are widely used for targeted drug delivery and as contrast agent. For these applications they are conjugated with various drugs, peptides, PEG, and DNA fragments.\(^12\) The resultant gold Nanoparticle conjugates have wide application in biomedicine and biosensing fields. For instance, conjugation of 6-Mercaptopurine with gold Nanoparticles leads to enhancement of anitproliferataive effect of this anti-leukemic drug.\(^13\) Also, gold Nanoparticles can act as immuno-potentiators when conjugated to various peptides like amyloid growth inhibitory peptide (AGIP).\(^14\)

Gold Nanoparticles have emerged as a promising weapon in the ongoing fight against cancer. They are being investigated as drug carriers, contrast agents, photo thermal agents and radiosensitizers.\(^15\) Zhao et al. worked on copper-64-alloyed gold nanoparticles and found that this unique alloyed nanostructure demonstrated higher passive tumour targeting and contrast ratios in the in vivo studies on mice. These results were contributed to the increased radiolabeling stability, superior targeting efficiency and improved diagnostic accuracy.\(^16\)

Thus the modification and funcionalization of GNP with different biological molecules (drugs, antibiotics, genes, peptides, plasmonic vesicles etc) can open new doors for efficient diagnosis and treatment of cancer.\(^17\)

Gold Nanoparticle conjugates can be prepared by any of the two methods, i.e. passive absorption or covalent coupling via a crosslinker. Passive
absorption is a simpler preparation process but it does not provide permanent attachment and the molecules may desorb from the surface over time. On the other hand, use of covalent coupling method permanently immobilizes the molecules of interest to the functionalized particles. By making use of chemical linkers (e.g., EDC/NHS) that react with certain groups on the molecules, covalent coupling becomes more specific and controllable than passive absorption and the number of covalently conjugated ligands can be optimized to particular application.

3. Gold nanoparticle conjugates for gene therapy:
Gene therapy has emerged as a promising way to treat number of diseases, ranging from inherited disorders to acquired conditions and cancer. Considering the central role of nucleic acids in the biological systems, adoption of ways to deliver wide variety of oligonucleotides such as plasmids, double stranded DNA (dsDNA), single stranded DNA (ssDNA) and single stranded RNA (ssRNA) can have tremendous therapeutic benefits. But the inability of these molecules to enter cell, due to the highly negative charge on them, calls for the use of some delivery vehicle. Also, these molecules were susceptible to degradation by proteases. Keeping this in mind, the past few years saw great research in the field of gold Nanoparticles mediated delivery of DNA and RNA. This delivery system of gold Nanoparticle conjugates has emerged as an innovative and feasible method for gene therapy.

3.1 Mechanism of Action:
It has been experimentally proven that upon exposure of gold Nanoparticles to cell culture, there occurred significant serum protein binding to the surface of Nanoparticle. This protein binding increased the cellular uptake of gold Nanoparticle conjugate. By varying the amount of bound DNA molecule per particle, it was found that increased levels of bound DNA led to increased serum protein binding to Nanoparticle surface, which further led to increased internalization of the gold Nanoparticle conjugate. Mirkin et al. carried out experiments, and conjugated citrate-stabilized spherical gold Nanoparticles with a dense layer of ssDNA molecules. This conjugate was internalized efficiently by cells and it was resistant to degradation by proteases. Binding of complementary DNA to the grafted ssDNA was strengthened due to the dense packing of DNA around the Nanoparticles which led to increased efficacy of the drugs in gene silencing applications.

This system is an example where the gold Nanoparticles are used not only for the facilitation of drug delivery but also for functioning as a critical part of the drug action.

In the experiments carried out by Mirkin et al. it was shown that in co-binding the gold Nanoparticle ssDNA conjugate with nuclear targeting peptides such as TAT or NLS, there occurred an increase in the perinuclear delivery of Nanoparticle conjugates. Mirkin et al. also conjugated anti-firefly luciferase siRNA to gold Nanoparticles and studied their gene knockdown potential compared to standard cationic lipid transfection agents. The result was found to be an eightfold increase in half-life of the conjugate as compared to molecular RNA. This increase was credited to the tight packing of the siRNA due to its covalent conjugation to the gold Nanoparticle which led to increase in its serum stability.

In certain cases where release of DNA from the delivery vehicle was desired, non-covalent interactions were used to bind the DNA to the gold Nanoparticle. For instance, functionalizing gold Nanoparticle to carry positive charge to which DNA can be electrostatically adsorbed.

Using light-responsive gold Nanoparticles is another way, wherein laser irradiation at appropriate frequency led to generation of “hot” electrons that triggered release of DNA covalently attached to a Nanoparticle.

Chen et al. used femtosecond laser irradiation to trigger the release of Enhanced Green Fluorescent Protein (EGFP) plasmid DNA attached to gold nanorods.
Until now the carriers used in clinical trials were recombinant viruses, wherein the gene of interest is covalently inserted into viral genome. But this caused severe immune responses and insertional mutagenesis in patients leading to carcinogenesis, germ-cell-line alteration or death. This prompted the development of nonviral delivery systems like chitosan, polyethylenimine (PEI) etc.\textsuperscript{12, 20}

3.2 chitosan-GNP conjugates:
DNA vaccination can be used for preventing numerous diseases but its efficiency is slow due to low immunogenicity. Low immunogenicity is due to poor delivery of DNA to antigen presenting cells (APCs) and rapid inactivation caused by DNase. These problems can be overcome by use of chitosan gold Nanoparticles conjugates as DNA vaccine delivery system.\textsuperscript{12, 21}

The transfection efficiency of chitosan depends on their molecular mass. For instance, the conventional high molecular weight chitosans are greatly efficient, but their poor physical properties like large aggregated shapes, low solubility at neutral pH, high viscosity at concentration used for in vivo delivery and a slow dissociation and release of plasmid DNA, lead to slow onset of action. Also the chitosans with an average molecular mass of 102kDa form very weak complexes with DNA, resulting in physically unstable polyplexes. These observations show that the two important characteristics, i.e. the stability of the Chitosan-GNP (gold Nanoparticles) conjugate in the biological milieu and the release of plasmid for expression should be balanced for an efficient DNA delivery system.\textsuperscript{12} Zhou et al. conjugated low molecular weight chitosans with an average molecular mass of 6kDa (Chito6) to GNPs. The resultant Chito6-GNPs conjugates formed stable complexes with plasmid DNA. This Chito6-GNP/DNA complex was then administered to mice by i.m. route and the immune responses induced were compared with the immunization with naked DNA. The results showed that the Chito6-GNP/DNA conjugate system protects the DNA from degradation and provide a fast release of DNA.\textsuperscript{12}

In order to assess the relative ability of this DNA vaccine technology to prime antibody and T-cell responses, Zhou et al. immunized mice with HBsAg DNA. The chito6-GNP significantly enhanced antibody responses to HBsAg over naked DNA at all the evaluated points. It was also shown that in contrast to naked DNA, Chito6-GNPs conjugates induced potent cytotoxic T lymphocyte responses at low dose.

Thus Chito6-GNPs have emerged as potent delivery systems for DNA vaccines and are capable of inducing enhanced humoral (10-fold) and cellular responses (100–1000-fold) after i.m. immunization with HBs Ag plasmid.\textsuperscript{12}
3.3 Polyethylenimine-GNP conjugates:
Polyethylenimine is a synthetic “proton-sponge” polycation which is used for transfection of DNA. The gold nanoparticles are covalently attached to branched polyethylenimine as vectors for the delivery of plasmid DNA. PEIs are preferably used as compared with other nonviral vectors because of its low cost and amenability to diverse and selective chemical modifications. The efficiency of this conjugate can be augmented by complexion with hydrophobic dodecy-PEI2.

4. Gold nanoparticle conjugates for bactericidal applications:
Many antibiotic compounds have been covalently attached to gold Nanoparticles to form gold nanoparticle-drug conjugates. These conjugates improve the efficacy of the drug molecule. Table 2 shows list of various drugs conjugated with GNPs and the type of bacteria against which it was tested successfully.

<table>
<thead>
<tr>
<th>Drug/treatment</th>
<th>Attachment</th>
<th>Tested against</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>Thiol</td>
<td>VRE, gram-negative</td>
</tr>
<tr>
<td>Vancomycin/photothermal</td>
<td>Thiol</td>
<td>Gram-positive, gram-negative, VRE, MRSA, PDRAB</td>
</tr>
<tr>
<td>Ampicillin, streptomycin, kanamycin</td>
<td>Amine (putative)</td>
<td>Gram-positive, gram-negative</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>Amine</td>
<td>Gram positive, gram negative</td>
</tr>
<tr>
<td>Touluidine blue O/photosensitization</td>
<td>Thiol</td>
<td>Gram-positive</td>
</tr>
</tbody>
</table>

4.1 Non-covalently bound antibiotics:
Simple procedures like mixing of citrate-capped gold Nanoparticles with an antibiotic or reduction of gold chloride in presence of the antibiotic resulted in non-covalent bonding between the two. This bonding was seen as aggregation and was followed by colour change from red to blue/purple. This conjugation due to the presence of amino group on the drug molecule has been proved to enhance bactericidal activity.

In some studies it was proved that aggregation may hinder the enhancement of action. Hence the antibiotic-GNP conjugate should be functionalized in a way that prevents aggregation and leads to a high surface coverage on the surface of Nanoparticles.

Another problem arising is the mixing of gold nanoparticle to molecules containing multiple amino groups. Using the drug molecules containing single amino group per molecule reduces aggregation. In the experiment carried out by Rai et al., a second-generation cephalosporin- Cefaclor, containing single amino group was used to form GNP conjugates. There was no aggregation and the conjugate was stable. The conjugate showed an increased bactericidal activity, in comparison to free Cefaclor, in both gram-positive and gram-negative bacteria and a decreased degradation than the free antibiotic drug.

4.1.1 Mechanism of action:
The conjugation with gold nanoparticle increased the cell penetration of the antibiotic into the gram-negative bacteria. It further enhanced the antibiotic action either by amplification of cell membrane damage by the drug or by aiding the drug in disruption of bacterial DNA.

4.2 Covalently bound antibiotics:
The antibiotic-gold nanoparticle conjugate may be formed by thiol-gold linkages. It involves attaching a thiol moiety to functionalize the gold surface. But thiols bound to gold surface are capable of exchanging with the thiols present in the solution. Rotello et al. demonstrated that this peculiar character can be used for drug release due to high levels of glutathione present in many types of cells.

Gu et al. conjugated 5nm GNP with vancomycin using phase transfer method. This vancomycin-GNP conjugate was found to be effective against a variety of Vancomycin-resistant enterococci (VRE), some gram negative bacteria like E.coli. It was even effective against all the bacterial strains against which vancomycin alone were ineffective.
4.2.1 Mechanism of action:
Multivalent binding of nanoparticles occurs wherein multiple vancomycin moieties bind to the terminal D-Ala-D-Ala moieties on the bacterial cell. This multivalent membrane binding enhances bactericidal activity of vancomycin.\textsuperscript{10}

5. Gold nanoparticle conjugates for anticancer properties:
The various drugs like cisplatin, doxorubicin, paclitaxel used for cancer treatment leads to serious systemic side effects due to their non-specificity. Also their effect is decreased over time due to less cellular uptake of these drugs into cancerous cells. Therefore, the current therapeutic strategy includes the use of gold nanoparticles as delivery agents for anti-cancer drugs.\textsuperscript{1,2}

Attachment to the gold nanoparticle leads to increased specificity and increased toxicity of the drug conjugate to the cancer cells as compared with free drug. This can be done either by passive targeting or by active targeting. While the process of passive targeting includes the enhancement of permeability and retention effect (EFR) based on the size of the gold nanoparticle conjugate, the active targeting uses antibodies or other targeting moieties.\textsuperscript{26} The various drugs that can be conjugated with gold nanoparticle are discussed below.

5.16-mercaptopurine-GNP conjugate:
Belonging to the antimetabolite class of anti-cancer drugs, 6-Mercaptopurine is a purine antagonist which is converted in the body to the corresponding monoribonucleotides.

These monoribonucleotides inhibit the conversion of inosine monophosphate to adenine and guanine nucleotides. There also occurs the feedback inhibition of de novo purine synthesis. It is widely used for the treatment of leukemias, choriocarcinoma, inflammatory bowel disease and systemic lupus erythematosus. Due to poor absorption of the drug by oral route, intravenous route was used for the administration of 6-MP. However, the drug has short plasma half-life due to its rapid elimination through the renal system. This leads to diminished effect of the drug.\textsuperscript{27}

In the renal system, very small particles are filtered off and the very large particles are removed by the reticular endothelial system (RES). Keeping in mind the filtration and RES systems, there must be an optimum particle diameter that will prevent renal filtration and will avoid immediate capture by the RES. This optimum size will correspond with maximum residence time in the blood and enhanced therapeutic effect.\textsuperscript{13}

6-Mercaptopurine is a thiol which can be easily loaded onto the surface of gold nanoparticles via sulfur-gold bonds. Podsiadlo et al. evaluated the growth-inhibitory effect of 6-MP against K-562 human chronic myeloid leukemia cells. They kept pure 6-MP and citrate stabilized GNPs as controls. The results showed the increased inhibition efficiency of 6-Mercaptopurine-GNP over the free drug. The inhibitory effect of the drug conjugate was stronger than the plain solution of 6-MP in the range between $1.8 \times 10^{-7}$ and $1.8 \times 10^{-6}$ M.\textsuperscript{13}

\textbf{FIG. 2: COMPARATIVE INHIBITION OF K-562 HUMAN CHRONIC MYELOID LEUKEMIA CELLS BY DIFFERENT DERIVATIVES OF GNPS AND 6-MPR. (A) ANITPROLIFERTAIVE EFFECTS OF SUBSTANCES INDICATED BELOW THE DATA BAR AGAINST LEUKEMIA CELLS AFTER 72 H OF INCUBATION. CONCENTRATION OF 6-MPR IN SOLUTIONS IS1.8*10^{-6} M; CONCENTRATION OF Au(III) IS 2.5*10^{-7} M. (B-E) REPRESENTATIVE OPTICAL MICROSCOPY IMAGES OF CELL CULTURE DISHES TREATED WITH DIFFERENT DERIVATIVES OF GNPS and 6-MPR: (B) NEGATIVE CONTROL (NO 6-MPR), (C) CELLS TREATED WITH FREELY DISSOLVED 6-MPR; (D) CELLS WITH CITRATE STABILIZED GNPs, and (E) CELLS TREATED WITH 6-MPR-GNPs.}\textsuperscript{13}
5.1.1 Mechanism of action:
Electrokinetic potential measurements of the 6-Mercaptopurine stabilized GNPs revealed them to be positively charged with Zeta potential to be +19mV. This positive charge of the 6-Mercaptopurine GNP strongly facilitates in its permeation through the negatively charged cell membranes. This is unlike the free drug which is neutral or slightly negative charged. Endocytosis also plays a major role in the intracellular movement of the drug conjugate and the its release. After Endocytosis, Nanoparticles being similar to various nanoscale biological objects such as proteins, virus and parts of other cells are are transferred to the lysosomes for digestion. In the lysosomes, the pH is more acidic, 4.8 than the surrounding cytosol or blood (pH 7). At this low pH of the lysosomes, the gold-sulfur bond is severed, thus the free drug is released which then inhibits the de novo synthesis of purines. The gold nanoparticles in the lysosomes are either coated by the proteins or are bound to the thiol group containing biomolecules, thus preventing the reabsorption of 6-MP onto the GNP surface.13

Thus conjugating 6-Mercaptopurine to Gold nanoparticles prevent the rapid elimination of the drug and increases the intracellular uptake of the drug. All these factors lead to enhanced therapeutic effect at nominal drug concentration which also decreases systemic side effects.

5.2 Methotrexate-GNP conjugate:
Belonging to the class of antimetabolites, Methotrexate is a folate antagonist which acts by inhibiting dihydrofolate reductase (DHFRase) enzyme, thus blocking the conversion of dihydrofolic acid (DHFA) to tetrahydrofolic acid (THFA). THFA is an important coenzyme required for one carbon transfer reactions in de novo purine synthesis and amino acid interconversions. It is used for treatment of various neoplasms, rheumatoid arthritis, and psoriasis and is also used as an immunosuppressant. 27

But there are various mechanisms by which the cancer cells become resistant to Methotrexate; these include, decreased influx of the drug through the reduced folate carrier and folate receptor and increased efflux of the drug from the cancer cells. Such mechanisms lead to decreased therapeutic action of Methotrexate. Also, when Methotrexate is locally administered in soluble form, it is rapidly absorbed through the capillaries into the blood circulation. This may also account for therapeutic failure in patients. Therefore, Methotrexate-GNP conjugates were introduced to help retain the drug within cancer cells for longer duration and to alter its pharmacokinetic properties. Methotrexate bind to the gold nanoparticle via carboxyl group (-COOH) to form Methotrexate-GNP complex.28

5.2.1 Mechanism of action:
The Methotrexate-GNP conjugate acts a ligand for the folate-binding protein expressed on the surface of the cell membrane and is thus internalized. Also, the gold nanoparticles interact with various cell membrane proteins that facilitate the internalization of the conjugated drug. Third mechanism accounting for increased influx of the drug into cancer cells is gold nanoparticles mediated Endocytosis. All these mechanisms together account for increased accumulation of intracellular Methotrexate, thus leading to dramatic improvement of its growth inhibitory effects.

In angiogenesis, a key event during tumour formation, vascular endothelial growth. Factor (VEGF) plays a vital role. In the experiments conducted by Chen et al., they demonstrated that gold nanoparticle binds to VGF and inhibit its angiogenic activity. Thus the “concentrated effect” of Methotrexate-GNP conjugates results in higher cytotoxic effect. 28

5.2.2 Methotrexate for psoriasis treatment:
Methotrexate can be used systemically for treatment of psoriasis. But its topical application to treat the disease is of limited use due to insufficient percutaneous penetration.

This insufficient penetration of the drug can be accounted due to limited passive diffusion, high molecular weight and dissociation at physiological pH. Use of gold nanoparticles for the topical delivery of the drug has emerged as a promising drug delivery system. Bessar et al. carried out in vivo testing on mice skin and found that the delivery of methotrexate in the epidermis and dermis is enhanced using Methotrexate-GNP
conjugate. Also, the GNP were found to be absent in epidermis and dermis, stating that these layers do not retain GNP. Thus GNP conjugate can be used as non-toxic carrier for the satisfactory percutaneous absorption of methotexate and can help in topical treatment of psoriasis. 29

5.3 doxorubicin-GNP conjugate:
Doxorubicin is an antitumour antibiotic. It enters the nucleus and causes break in the DNA strands by activating topoisomerase II and generating quinine type free radicals. Recently, doxorubicin was covalently bound to gold nanoparticles to treat drug resistant breast cancer. 27

5.3.1 Mechanism of action:

The gold nanoparticles helped in Endocytosis of the drug into the otherwise resistant cancer cells. The doxorubicin-GNP conjugate is stable in the pH of blood and cytoplasm (pH 7.2). But in the acidic pH of endosomes, the drug is released from the conjugate. This leads to increased concentrations of the drug in the cancer cells. The drug then enters the nucleus and exhibits its cytotoxic effects.

This overcomes the efflux of doxorubicin from the cancer cells and diminishes the drug resistance of cells. Therefore, gold nanoparticle bound doxorubicin is many times more cytotoxic than the free drug. 30

Chen et al. also conjugated doxorubicin with GNP via thio-gold bond by using a peptide substrate. In vivo studies on tumor bearing mice showed that over-expressed protease in tumor cells led to the rapid release of drug from the GNP and higher tumor inhibitory action. 31

5.4 platinum based GNP conjugates:
Cisplastin is a platinum coordination complex that is hydrolysed intracellularly to produce a highly reactive moiety which causes cross linking of DNA. It is used to treat testicular, ovarian, cervical and oesophageal cancer. But it has serious side effects like kidney toxicity, irreversible peripheral nerve damage etc. 27 In order to improve the delivery and therapeutic effect of the drug, DNA-gold nanoparticle complex is used as a drug delivery vehicle. In place of platinum(II) compounds, platinum(IV) complexes are used as prodrug to minimize the side effects. The inertness of Pt(IV) complexes provide an attractive alternative to the Pt(II) drug.
In the intracellular milieu, Pt(IV)-DNA-GNP conjugate is reduced to release a cytotoxic dose of cisplatin. After the conjugate is internalized, cisplatin enters nucleus and forms 1,2-d(GpG) intrastrand cross-links with DNA.\(^{32}\)

FIG. 4: SCHEMATIC REPRESENTATION OF CELL UPTAKE AND CISPLATIN RELEASE FROM GOLD NANORODS CONJUGATED WITH PT(IV) PRODRUG.\(^ {10}\)

5.5 paclitaxel-GNP conjugate:
Paclitaxel (PTX) is a cell cycle specific chemotherapeutic drug that kills only actively dividing cells. It basically acts on the M phase and promote tubulin assembly into microtubules. Thus the microtubules are stabilized and this leads to inhibition of normal dynamic reorganization of microtubule network important for interphase and mitotic functions. It is the most popular anti-cancer drug for used for the treatment of breast, ovarian and lung cancer.\(^ {27}\) But the drug lacks specificity, leading to serious side effects and diminished therapeutic effect. It also has low solubility in water. Thus need of the hour led to the use of paclitaxel-gold nanoparticle conjugate to improve the specificity of the drug for cancer cells.

Hwu et al. synthesized paclitaxel-functionalized at C-2 position using a phosphodiester linkage and demonstrated that the incorporation of the phosphodiester moiety in paclitaxel-GNP conjugate helped to improve selective targeting using the GNP. The drugs containing a phosphate unit preferentially interacts with cancer cells. Also, in cancer cells dephosphorylation occurs more easily than in normal cells. This hydrolysis of phosphodiester moiety by phosphodiesterase helps in the liberation of free nanoparticle from the gold nanoparticle.\(^ {33}\)

Mirkin et al. synthesized oligonucleotide-GNP conjugates with paclitaxel. Thus the paclitaxel, which is otherwise insoluble in aqueous phase, now gets solubilized. This GNP-DNA-paclitaxel conjugate was easily internalized in the cancer cell and demonstrated enhanced cytotoxic effect. It was shown to be toxic to even paclitaxel-resistant cell lines due to increased uptake and decreased efflux.\(^ {10}\)

Heo et al. functionalized the surface of GNP with paclitaxel, PEG, biotin and rhondamine B linked cyclodextrin. In this conjugate system, PEG act as anti-fouling agent for diagnostic purpose. Biotin binding receptors are over expressed on the surface of cancer cells to sustain their rapid proliferation, thus making biotin useful as cancer-specific targeting ligand. Paclitaxel is poorly soluble in water. To overcome this problem cyclodextrin is used as drug pocket as it improves the water-solubility of the drug. This GNP conjugate system can hence be used as a cancer cell specific theranostic agent.\(^ {34}\)

FIG. 5: SCHEMATIC ILLUSTRATION OF CANCER-TARGETING DELIVERY MECHANISM OF PACLITAXEL CONJUGATED GNP AS A THERANOSTIC AGENT.\(^ {34}\)
5.6 Other anti-cancer drugs conjugated with GNP:
5-Fluorouracil- Agastiet et al. synthesized fluorouracil-functionalized gold nanoparticles. They then irradiated the nanoparticles with 365nm UV light which led to controlled release of the drug, thus exerting its cytotoxic effect only after release from the gold nanoparticle surface. This lack of toxicity before photo-induced release of ligand is useful for targeted treatment of cancer. It releases the drug by passive targeting mechanism (EPR effect).10

Cancer-targeted drug delivery can also be enhanced by use of GNP-conjugated plasmonic vesicles. Song et al. produced bioconjugated plasmonic vesicles assembled from SERS-encoded amphiphilic gold nanoparticles. These plasmonic assemblies having hollow cavity can play wide role as delivery carriers for anticancer drugs and their labeling can be used to monitor intracellular drug delivery.35

6. Air plasma coupled with antibody conjugated GNP:
Ambient air plasma can kill cancer cells. To improve the selectivity of air plasma, antibody-GNP conjugates are used. The gold nanoparticles are conjugated with anti-FAK (focal adhesion kinase) antibodies (FAK-GNPs). FAK is overexpressed in numerous cancer cells. The increased expression of FAK is a crucial step for the survival, growth and metastasis of melanoma cells. Therefore, degradation of FAK can be utilized as a good tool for the selective melanoma therapy.36

6.1 Mechanism of action:
After translocation across the cell membrane, the FAK-GNPs bind to FAK. Then the irradiation of plasma gold nanoparticles leads to the deactivation of FAK. This increases the death rate of cancer cells by 74%.

Thus the use of antibody conjugated gold nanoparticles increased the cell death by plasma treatment by 5 times than when plasma treatment was used alone.36

7. Gold nanoparticles for diagnostic purposes:
Detection of tumour in organs forms an important part of diagnosis of cancer. It uses various technologies like ultrasound and optical tomography. Though they are provided at low cost, these technologies have various disadvantages, like ultrasound lacks discrete contrast between malignant and benign tumours and optical topography has poor resolution due to scattering of light in tissues. In the past few years, tremendous research has been done to highlight the potential applications of targeted gold nanoparticle conjugates for biomedical imaging. Till now these applications have been proposed for imaging of superficial tumours.37,38

7.1 GNP as optoacoustic contrast agent:
Preferential absorption of laser light by tumours is due to the inherent difference in their optical properties as compared to adjacent normal cells. This difference is due to the haemoglobin of blood. In advanced tumours abnormal angiogenesis occurs, leading to increase in blood content of tumours. This serves as endogenous contrast agent to optoacoustic tomography (OAT). But lack of ability to differentiate between tumour and normal cells in early stage paves way for the use of monoclonal antibody conjugated gold nanoparticle to serve as exogenous contrast agent in OAT. This method is widely used for the diagnosis breast, ovary, prostate, bladder, kidney and lung cancer.39

7.1.1 Mechanism of action:
Copland et al. conjugated gold nanoparticles to humanized IgG antibody (Herceptin) which is
currently used for treatment of breast cancer. IgG antibodies usually bind to the extracellular domain of HER2 tyrosine kinase receptors. These receptors are over expressed in breast, ovary, prostate and lung cancer. Hence when optoacoustic imaging was done, the GNP conjugates act as contrasting agents for the diagnosis of various tumours in their early stage. The studies showed that the increase of the optoacoustic signal amplitude within a tumour is proportional to the number of accumulated gold nanoparticles.39

7.2 polymer conjugated GNP as antifouling agent:
Gold nanoparticles can be conjugated to polyethylene glycol (PEG) by the reaction of thiol group of PEG and the gold nanoparticle surface. Conjugation to PEG imparts antifouling properties to the gold nanoparticles thus extending their lifetime in bloodstream. Therefore, PEG-GNP conjugate is used as CT contrast agent. Unlike iodine based CT scan imaging which allows very short imaging due to rapid clearance by kidneys, PEG-GNP conjugates act as better agents for CT scan.40

Kim et al. examined to feasibility of PEG-GNP conjugate as CT contrast agent. They performed the blood pool imaging of rats following injection of the conjugate.40 The GNP-enhanced CT image with good contrast is shown in Fig.7.

On carrying out the quantitative analysis of CT values, it was found that PEG-GNP conjugate stays in the blood for the appreciable time of 4 hours, thus providing enough time for the imaging.

7.3 Transferrin conjugated GNP:
Gold nanoparticles are widely used for the diagnosis of cancer cells. Introduction of specificity to the GNP can help in selective and enhanced targeting. Studies have shown that conjugation of transferrin molecules on the gold nanoparticle surface can enhance the cellular uptake of GNP into the cancer cells by six times.

7.3.1 Mechanism of action:
Transferrin receptors are the surface glycoproteins related to cell proliferation. These receptors play a vital role in the transportation of iron for the synthesis of haemoglobin. Because of the higher iron demand of cancer cells for faster cell growth and division, transferrin receptors are present in abundance on the surface of cancer cells.

Binding of GNP with transferring molecules increases their specificity for cancer cells. This increased specificity helps in reducing the laser power effective for therapy from 1600W/cm² to 7W/cm² as was demonstrated by Li et al. Therefore, the transferrin-conjugated gold nanoparticles can be potentially used for the cancer diagnostics and therapy.37

7.4 Anti-egfr antibody conjugated GNP:
Gold nanoparticles can resonantly scatter visible and near infrared light. This property is sensitive to the size, shape and aggregation state of the gold nanoparticles and occurs due to excitation of surface Plasmon resonances. This property of GNP is utilized by conjugating it with anti-EGFR antibodies and visualizing the specific binding of this conjugate to the malignant cells. This binding with malignant cells gives relatively sharper SPR absorption band compared to that observed with normal cells.41, 42 These anti-EGFR conjugated GNP have also been used for photothermal treatment and drug delivery.43

8. GNP conjugates in molecular immunology:
An adjuvant which is to be used as vaccine for enhancing immune response should improve
antigen presentation and increase co-stimulatory and cytokine production. Gold nanoparticles conjugated to various peptides can act as immune-potentiators and activate macrophages which further react against pathogenic organisms. The peptide molecules like the amyloid growth inhibitory peptide (AGIP) and the sweet arrow peptide (SAP), which were otherwise invisible to the immune system, are detected in presence of GNP.\textsuperscript{14}

8.1 \textbf{Mechanism of action:} The recognition of AGIP and SAP by the macrophages in the presence of GNP is mediated by TLR4, a pattern recognition receptor. This leads to the induction of pro-inflammatory cytokines such as TNF-\(\alpha\), IL-\(\beta\) and IL-6 and nitric oxide synthase. This macrophages activation by the peptides in the presence of gold nanoparticles paves the way for the use of GNP conjugates as immune-potentiators in molecular immunology.\textsuperscript{14}

\textbf{CONCLUSION:} In the past few years, tremendous research has been done on nanoparticles as vehicle for drug delivery and in diagnostics. The gold nanoparticles find extensive use due to their large surface area and unique optical properties. Difference in shape and size impart unique electric and magnetic properties to gold nanoparticles, which has led to many biochemical applications. Various conjugation products can be made by functionalizing gold nanoparticles using either passive absorption or covalent coupling via a cross-linker method. The advantages of gold nanoparticle conjugates as drug delivery vehicles have led to breakthrough research in the field of medicine.

The use of gold nanoparticle conjugates in gene vaccination has overcome the side-effects of viral vectors in gene delivery and it also holds the key to treat various diseases. Also, the conjugation of various anti-bacterial drugs to gold nanoparticles has proved to be good therapeutic agent for killing drug resistant microorganisms, like VRE. Gold nanoparticles conjugation with anti-cancer drugs has enhanced drug selectivity for cancer cells and hence helped outdo the serious cytotoxic effects of these drugs. In the recent years, Gold nanoparticles conjugates have also been used as contrast agents in the imaging of various tumours using peptides and antibiotics. Thus gold nanoparticle conjugates hold a promising future in the field of biomedicine and diagnostics.

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\textbf{REFERENCES:}

13. Podsiadlo Paul, Sinani VA, Bahng JH, Kam NW, Lee Jungwoo, Kotov NA: Gold Nanoparticles enhance the